A DIFFERENCE IN THE BREAKDOWN OF PHOSPHATIDYLINOSITOL IN NORMAL AND SV40 TRANSFORMED MOUSE FIBROBLASTS

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Summary: Pulse chase experiments have shown that four reactions of phospholipid metabolism depend on cell population density in normal cells. Three of these reactions are influenced by population density also in tumor cells. Only the breakdown of phosphatidylinositol is not affected.

Introduction: Kinetic studies have shown that the metabolism of at least three phospholipids is affected when normal embryonic mouse fibroblasts are transferred from a state of low to a state of high cell population density (1).

When sparsely growing cells, prelabelled with ^{32}P-orthophosphate and 1(3)-^{3}H-glycerol are shifted to a state of high population density by addition of unlabelled cells (2×10^7 cells/10 cm petri dish) the following phenomena are observed:

1. an inhibited uptake of ^{32}P into phosphatidylethanolamine,
2. a slower loss of ^{32}P from phosphatidylinositol (1) as well as from phosphatidylcholine and
3. an inhibited reutilization of the glycerol moiety of degraded phosphatidylinositol for the formation of phosphatidylinositol.

Such kinetic studies have now been extended to SV40 transformed mouse fibroblasts derived from the same strain of mice (STU). The results of these experiments reveal a distinct difference between normal cells and tumor cells in the metabolism of phosphatidylinositol.

Results and Discussion: Exponentially growing SV40 transformed mouse fibroblasts (STU-261) were labelled for 12 hours with ^{32}P-orthophosphate and ^{3}H-glycerol following the procedures described previously (1). During the subsequent chase period a prolonged uptake of radioactivity into phosphatidylethanolamine is observed as compared to phosphatidylcholine and phosphatidylinositol (Fig.1 full circles). When however, in such cultures an intimate cell to

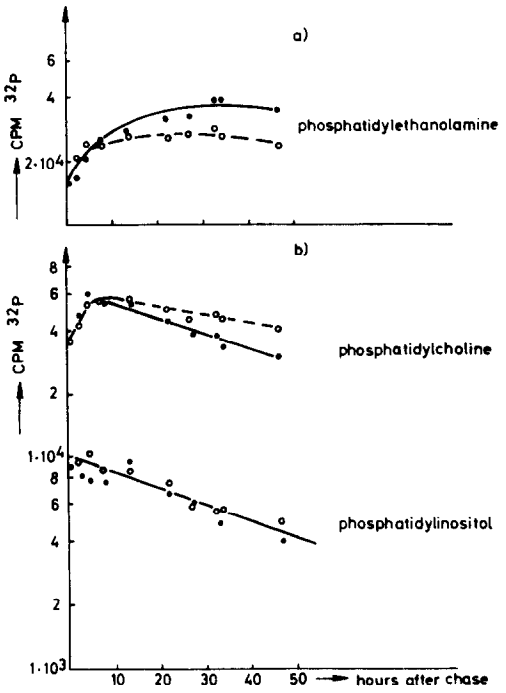

Fig. 1 a) The ^{32}P-content of phosphatidylethanolamine of exponentially growing (• — •) and densely packed (o — o) STU-261 cells. b) The ^{32}P-content of phosphatidylcholine and phosphatidylinositol of growing (• — •) and densely packed (o — o) STU-261 cells.

In samples at high cell population density the absolute amount of ^{32}P in the total phospholipid fraction was always found to be higher than in samples from growing cells caused by a smaller loss of radioactive cells during the isolation procedures. The data compare equal amounts of radioactivity present in the cellular phospholipids under both growth conditions.

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cell contact is established by addition of unlabelled cells this prolonged incorporation of ^{32}P into phosphatidylethanolamine no longer occurs.

Radioactive phosphorus is lost from phosphatidylcholine beginning about 6 hours after the chase in exponentially growing cells and in confluent cultures. The rate of loss, however, is significantly lower in confluent cells. This holds also for normal cells (to be published and Table 1).

No difference is detectable in the breakdown of phosphatidylinositol in tumor cells at high and low cell population density. This finding is in variance with the results obtained in similar experiments with normal mouse fibroblasts(1).

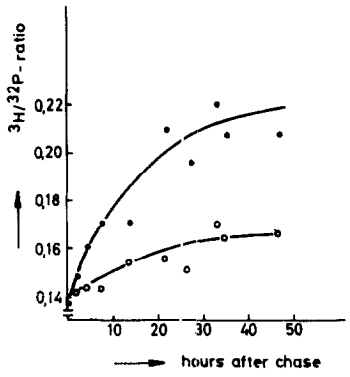

Fig. 2 The $^3H/^{32}P$-ratio of phosphatidylinositol isolated from prelabelled cells: (• — •) low cell population density, (o — o) high cell population density.

In exponentially growing normal cells (1) as well as in the tumor cells studied by us (2) the ratio of tritiated glycerol to radioactive phosphorus increases in phosphatidylinositol only. This phenomenon is not significant in confluent cultures (Fig.2).

In two additional experiments the findings depicted in Figs. 1 and 2 were confirmed. The results of the three experiments are summarized in Table 1 and compared with results obtained in two experiments with normal cells. As can be seen from the doubling time of the total lipid phosphorus, serving as indicator for cell growth (3), normal cells cease to grow when cell to cell contact is established. Tumor cells, however, continue to grow under these conditions yet somewhat slower. Release of ^{32}P from phosphatidylcholine as measured by the half life of the incorporated absolute radioactivity proceeds less rapidly in normal cells as well as in tumor cells at high population density. This cell density dependent difference in the metabolism of phosphatidylcholine appears to be more pronounced in tumor cells. Under conditions of close cell to cell contact the incorporation of radioactivity into phosphatidylethanolamine as measured by the maximum uptake of ^{32}P-phosphate into this lipid is inhibited in normal and in tumor cells.

These two changes are consistently observed when cells are in close contact regardless whether the cells are growing or not. This suggests that these two changes are related to cell population density rather than to cell growth. Likewise density dependent is the $^3H/^{32}P$ ratio of phosphatidylinositol (Fig.2), indicating biosynthesis of phosphatidylinositol with a reutilisation of the glycerol moiety.

Table 1

Comparison of the metabolism of three phospholipids of normal and SV40 transformed mouse cells at indicated cell population densities

cells	doubling time of lipid phosphorus (hours)		release of ^{32}P from PC t/2 (hours)		maximum incorporation of ^{32}P into PE (CPMx10^{-4})		release of ^{32}P from PI t/2 (hours)	
	low	high	low	high	low	high	low	high
STU (normal)	50	>500	30	42	7.9	5.6	6	18
	38	>300	27	38	5.0	4.0	14	39
STU-261 (SV40)	17	33	40	80	3.6	2.9	40	43
	17	44	28	63	1.4	0.9	30	33
	13	40	40	68	2.8	2.0	25	25

The half life of the ^{32}P content of phosphatidylcholine (PC) and phosphatidylinositol (PI) was determined for the period from 10 to 40 hours after the chase assuming first order kinetics. PE = phosphatidylethanolamine

The release of ^{32}P-phosphate from the phosphatidylinositol was found to proceed most rapidly in exponentially growing normal cells. When these cells stop to divide the release of ^{32}P from this lipid proceeds about three times slower. In growing tumor cells (STU-261) this reaction is slower as compared to normal cells. A dependency on cell population density of this reaction is not detectable in tumor cells. We therefore assume that the cleavage of phosphatidylinositol is in some way linked to the regulation of cell growth.

During the cleavage of phosphatidylinositol inositol-1-phosphate as well as cyclic inositol-1,2-phosphate are released as first described by Dawson et al. (4,5) and more recently by Lapetina and Michell (6,7). The degradation of phosphatidylinositol was found to occur also in cell homogenates. We, therefore, have studied this reaction in homogenates of growing mouse fibroblasts and of two

Table 2

Degradation of phosphatidylinositol by homogenates of exponentially growing normal mouse cells and two SV40 transformed mouse cell lines

cells	incubation (hours)	water soluble ^{32}P (CPMx10^{-3}) per mg protein	per µmol lipid phosphorus	ratio of radioactive I-1,2-P/ I-1-P +
STU (normal)	1	0.97	7.0	0.70
	3	1.24	10.1	0.27
STU-261 (SV40)	1	0.35	3.5	1.01
	3	0.66	5.9	0.62
STU-51A/ 232B (SV40)	1	0.46	5.1	1.30
	3	0.89	7.4	0.37

The incubation was performed according to Lapetina and Michell (8) in 0.2 ml of homogenate containing 7-8 mg protein and 17 x 10^3 CPM of ^{32}P-phosphatidylinositol (95% purity, isolated from labelled tumor cells). The reaction was stopped by the addition of 4.0 ml chloroform/methanol 2/1 and short ultrasonication to extract the lipids. After centrifugation and removal of the extract the residue was sonicated with 0.8 ml of water. Combination of the water- and the chloroform/methanol extracts resulted in two layers. Radioactivity was measured in aliquots of both layers (lower fraction: undegraded phosphatidylinositol; upper fraction: water soluble split products). The upper layer was evaporated to dryness and the residue was then dissolved in a small volume of water and chromatographed (8). Radioactivity was determined with the Packard Radiochromatogram Scanner.

+ I-1,2-P : cyclic inositol-1,2-phosphate; I-1-P : inositol-1-phosphate.

tumor cell lines (STU-261 and STU-51A/232B) as described in the legend of Table 2. In all cases the recovery of phosphatidylinositol and its water soluble split products was at least 95% on the basis of the radioactivity present at the onset of the experiment. In all three experiments the degradation of phosphatidylinositol proceeds

for at least three hours. In homogenates from normal mouse fibroblasts the degradation is more rapid than in homogenates from tumor cells. This holds true whether the amount of water soluble radioactive phosphorus is expressed per mg protein present in the homogenate or per µmol of lipid phosphorus in the sample of isolated lipids. The results are not unexpected considering the results obtained from kinetic studies in intact cells (see Table 1).

By paper chromatography according to Dawson and Clarke (8) it is possible to separate the water soluble split products of phosphatidylinositol degradation. In our experiments more than 80% of the radioactivity applied was associated with cyclic inositol-1,2-phosphate and inositol-1-phosphate. Cyclic inositol-1,2-phosphate used for reference purposes was synthesized according to Pizer and Ballou (9). The results given in Table 2 show that the ratio of cyclic inositol-1,2-phosphate to inositol-1-phosphate in all cases decreases with time of incubation. In tumor cells this ratio seems to be consistently higher than in normal cells.

Of four reactions of the phospholipid metabolism investigated in normal cells and tumor cells at low and high cell population densities only one is different in the tumor cells, i.e. the breakdown of phosphatidylinositol is not inhibited by close cell to cell contact in tumor cells. Furthermore, this breakdown appears to proceed more rapidly in growing normal cells than in growing tumor cells as indicated by results obtained from intact cells and cell homogenates.

Phosphatidylinositol metabolism has attracted increasing attention in connection with cell stimulation (for review see 10).

According to experiments with cerebral cortex by Lapetina and Michell (7) it appears that enzymes of phosphatidylinositol breakdown are present in the plasma cell membranes. It is a well established fact that most or all tumor cells have altered cell surfaces, detectable by immunological methods (11). It is not unreasonable to assume that such topological changes could alter the regulation of phosphatidylinositol metabolism.

It remains to be investigated whether our observations can be extended to other tumor cell systems.

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